

the ext nt revisions utilize the essence of the present invention, each would naturally fall within the breath or protection encompassed by this path. This is particularly true for the present invention since its basic concepts and understandings are fundamental in nature and can be broadly applied. It is also particularly true since the present invention involves a number of potentially independent features which may be combined in synergistic ways for particular applications.

## V. CLAIMS

### I. claim:

1. A method of creating a droplet from a jet of a flow cytometer comprising the steps of:
  - a. establishing a nozzle volume;
  - b. introducing a flow of sheath fluid into said nozzle volume;
  - c. introducing a flow of a substance within said sheath fluid in said nozzle volume;
  - d. establishing an oscillator coupled to said nozzle volume;
  - e. applying an alternating voltage with an amplitude of less than one hundred millivolts to said oscillator;
  - f. allowing said sheath fluid to exit from said nozzle volume; and
  - g. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.
2. A method of creating a droplet from a jet of a flow cytometer as described in claim 1 wherein the amplitude of said alternating voltage is about ten millivolts.
3. A method of creating a droplet from a jet of a flow cytometer as described in claim 1 wherein said oscillator is established within said nozzle volume.

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A method of creating a droplet from a jet of a flow cytometer as described in claim 3 wherein said nozzle volume has a perpendicular cross sectional area and wherein said oscillator is established substantially throughout said perpendicular cross sectional area.

5. A method of creating a droplet from a jet of a flow cytometer as described in claim 3 wherein said oscillator is unidirectionally coupled to said sheath fluid.

6. A method of creating a droplet from a jet of a flow cytometer as described in claim 1, 4, or 5 and further comprising the step of continuously converging said sheath fluid within said nozzle volume.

7. A system for creating a droplet from a jet of a flow cytometer comprising:

- a. a nozzle container establishing a nozzle volume and having a nozzle exit;
- b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;
- c. a substance introduction port located within said nozzle volume;
- d. an oscillator to which said sheath fluid is responsive;
- e. an alternating voltage source having an alternating voltage amplitude of less than one hundred millivolts connected to said oscillator; and
- f. a free fall area below said nozzle exit and within which said droplet forms.

8. A system for creating a droplet from a jet of a flow cytometer as described in claim 7 wherein said alternating voltage amplitude is about ten millivolts.

8. A system for creating a droplet from a jet of a flow cytometer as described in claim 7 wherein said oscillator is within said nozzle container.

9. A system for creating a droplet from a jet of a flow cytometer as described in claim 9 wherein said nozzle container has a cap section and wherein said oscillator comprises a piezoelectric crystal contained within said sheath fluid and attached to said cap section.

10. A system for creating a droplet from a jet of a flow cytometer as described in claim 10 wherein said sheath fluid port introduces a sheath fluid, wherein said oscillator has an oscillator surface which faces said sheath fluid, and further comprising an interface material between said oscillator surface and said sheath fluid which consists essentially of a protective coating.

11. A system for creating a droplet from a jet of a flow cytometer as described in claim 9 wherein said nozzle container has a largest perpendicular cross sectional area and wherein said oscillator is located at said largest perpendicular cross sectional area and is substantially as large as said largest perpendicular cross sectional area.

12. A system for creating a droplet from a jet of a flow cytometer as described in claim 10 wherein said oscillator has an oscillator side and further comprising a spacer which maintains said oscillator side detached from said nozzle container.

13. A system for creating a droplet from a jet of a flow cytometer as described in claim 7 or 12 wherein said nozzle container continuously converges.

15. A system for creating a droplet from a jet of a flow cytometer as described in claim 14 wherein said converging nozzle container continuously converges from said sheath fluid port to said nozzle exit.

16. A system for creating a droplet from a jet of a flow cytometer as described in claim 14 wherein said converging nozzle container comprises:

- a. a nozzle body having an inner surface;
- b. a nozzle tip having an inner surface; and
- c. a seal located off of said inner surface of said nozzle tip and to which both said nozzle body and said nozzle tip are responsive.

17. A method of creating a droplet from a jet of a flow cytometer comprising the steps of:

- a. establishing a nozzle volume;
- b. introducing a flow of sheath fluid into said nozzle volume;
- c. introducing a flow of a substance within said sheath fluid in said nozzle volume;
- d. initiating an oscillation within said nozzle volume;
- e. allowing said sheath fluid to exit from said nozzle volume; and
- f. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.

18. A method of creating a droplet from a jet of a flow cytometer as described in claim 17 wherein said step of initiating an oscillation within said nozzle volume comprises the step of establishing an oscillator within said sheath fluid.

19. A method of creating a droplet from a jet of a flow cytometer as described in claim 17 wherein said oscillation passes

through a material interface and further comprising the step of minimizing the number of material interfaces which said oscillation must pass through.

A method of creating a droplet from a jet of a flow cytometer as described in claim 19 wherein said oscillation is created by an oscillator and wherein said oscillation passes through only a protective coating on said oscillator before imparting on said sheath fluid.

A method of creating a droplet from a jet of a flow cytometer as described in claim 19 wherein the flow of said sheath fluid has a primary flow direction and wherein said step of minimizing the number of material interfaces which said oscillation must pass through comprises the step of coupling said oscillation in substantially only said primary flow direction.

A method of creating a droplet from a jet of a flow cytometer as described in claim 17 and further comprising the step of directly transferring said oscillation to said sheath fluid.

A method of creating a droplet from a jet of a flow cytometer as described in claim 17, 18, or 22 and further comprising the step of unidirectionally applying an oscillation to said sheath fluid.

A method of creating a droplet from a jet of a flow cytometer as described in claim 23 and further comprising the step of directionally isolating said oscillation.

A method of creating a droplet from a jet of a flow cytometer as described in claim 17 and further comprising the step of directionally isolating said oscillation.

25. A method of creating a droplet from a jet of a flow cytometer as described in claim 24 wherein said step of isolating said scillation comprises the step of coupling said oscillation to said sheath fluid along only one plane.

27. A system for creating a droplet from a jet of a flow cytometer comprising:

- a. a nozzle container establishing a nozzle volume and having a nozzle exit;
- b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;
- c. a substance introduction port located within said nozzle volume;
- d. an oscillator within said nozzle container; and
- e. a free fall area below said nozzle exit and within which said droplet forms.

A system for creating a droplet from a jet of a flow cytometer as described in claim 27 wherein said sheath fluid port introduces a sheath fluid and wherein said oscillator comprises a piezoelectric crystal contained within said sheath fluid.

29. A system for creating a droplet from a jet of a flow cytometer as described in claim 27 wherein said sheath fluid port introduces a sheath fluid, wherein said oscillator has an oscillator surface which faces said sheath fluid, and further comprising an interface material between said oscillator surface and said sheath fluid which consists essentially of a protective coating.

31. A system for creating a droplet from a jet of a flow cytometer as described in claim 27 wherein said nozzle container comprises:

- a. a cap section;

- b. a nozzle body sealed to said cap section; and  
c. a nozzle tip having said nozzle exit situated thereon,  
wherein said nozzle tip is sealed to said nozzle body,  
and wherein said sheath fluid flows through said nozzle  
tip.

11. A system for creating a droplet from a jet of a flow cytometer  
as described in claim 27 wherein said oscillator has an  
oscillator surface which faces said sheath fluid and wherein  
said oscillator surface is planar.

12. A system for creating a droplet from a jet of a flow cytometer  
as described in claim 27 or 28 wherein said sheath fluid port  
introduces a sheath fluid and further comprising a coupling  
which is only planar and which couples said oscillator to said  
sheath fluid.

13. A system for creating a droplet from a jet of a flow cytometer  
as described in claim 32 and further comprising a directional  
isolator between said oscillator and said nozzle container.

14. A system for creating a droplet from a jet of a flow cytometer  
as described in claim 33 wherein said oscillator emits a  
predominant frequency and wherein said directional isolator is  
effective at said predominant frequency.

15. A system for creating a droplet from a jet of a flow cytometer  
as described in claim 33 wherein said oscillator has an  
oscillator side and wherein said directional isolator  
comprises a spacer which maintains said oscillator side  
detached from said nozzle container.

16. A method of creating a droplet from a jet of a flow cytometer  
comprising the steps of:

- a. establishing a nozzle volume;

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- b. introducing a flow of sheath fluid into said nozzle volume;
  - c. introducing a flow of a substance within said sheath fluid in said nozzle volume;
  - d. unidirectionally applying an oscillation to said sheath fluid;
  - e. allowing said sheath fluid to exit from said nozzle volume; and
  - f. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.

37. A method of creating a droplet from a jet of a flow cytometer as described in claim 36 wherein said step of unidirectionally applying an oscillation to said sheath fluid comprises the step of directionally isolating said oscillation.

38. A method of creating a droplet from a jet of a flow cytometer as described in claim 37 wherein said step of isolating said oscillation comprises the step of coupling said oscillation to said sheath fluid along only one plane.

39. A system for creating a droplet from a jet of a flow cytometer comprising:

- a. a nozzle container establishing a nozzle volume and having a nozzle exit;
- b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;
- c. a substance introduction port located within said nozzle volume;
- d. a free fall area below said nozzle exit and within which said droplet forms;
- e. an oscillator to which said sheath fluid is responsive; and

f. a unidirectional coupling which couples said oscillator to said sheath fluid.

A system for creating a droplet from a jet of a flow cytometer as described in claim 39 wherein said unidirectional coupling comprises a surface which is only planar.

A system for creating a droplet from a jet of a flow cytometer as described in claim 39 and further comprising a directional isolator between said oscillator and said nozzle container.

A system for creating a droplet from a jet of a flow cytometer as described in claim 41 wherein said oscillator emits a predominant frequency and wherein said directional isolator is effective at said predominant frequency.

A system for creating a droplet from a jet of a flow cytometer as described in claim 41 wherein said oscillator has an oscillator side and wherein said directional isolator comprises a spacer which maintains said oscillator side detached from said nozzle container.

A method of creating a droplet from a jet of a flow cytometer comprising the steps of:

- A. establishing a nozzle volume;
- B. introducing a flow of sheath fluid into said nozzle volume;
- C. continuously converging said sheath fluid;
- D. introducing a flow of a substance within said sheath fluid in said nozzle volume;
- E. allowing said sheath fluid to exit from said nozzle volume; and
- F. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.

45. A method of creating a droplet from a jet of a flow cytometer as described in claim 44 wherein said flow cytometer has a nozzle body and nozzle tip and wherein said step of continuously converging said sheath fluid comprises the step of establishing a smooth transition from said nozzle body to said nozzle tip.

46. A method of creating a droplet from a jet of a flow cytometer as described in claim 44 wherein said nozzle volume has a largest perpendicular cross sectional area and further comprising the step of applying an oscillation to said sheath fluid at said largest perpendicular cross sectional area.

47. A method of creating a droplet from a jet of a flow cytometer as described in claim 46 wherein said step of applying an oscillation to said sheath fluid is accomplished substantially throughout said largest perpendicular cross sectional area.

48. A method of creating a droplet from a jet of a flow cytometer as described in claim 44 and further comprising the step of establishing an oscillator within said sheath fluid.

49. A method of creating a droplet from a jet of a flow cytometer as described in claim 48 wherein said oscillator establishes an oscillation and wherein said oscillation passes through a material interface and further comprising the step of minimizing the number of material interfaces which said oscillation must pass through.

50. A method of creating a droplet from a jet of a flow cytometer as described in claim 49 wherein said oscillation passes through only a protective coating on said oscillator before imparting on said sheath fluid.

51. A method of creating a droplet from a jet of a flow cytometer as described in claim 49 wherein the flow of said sheath fluid has a primary flow direction and wherein said step of minimizing the number of material interfaces which said oscillation must pass through comprises the step of coupling said oscillation in substantially only said primary flow direction.

52. A method of creating a droplet from a jet of a flow cytometer as described in claim 48 and further comprising the step of directly coupling said oscillator to said sheath fluid.

53. A method of creating a droplet from a jet of a flow cytometer as described in claim 44, 48, 49 or 51 and further comprising the step of unidirectionally applying an oscillation to said sheath fluid.

54. A method of creating a droplet from a jet of a flow cytometer as described in claim 53 and further comprising the step of directionally isolating said oscillation.

55. A method of creating a droplet from a jet of a flow cytometer as described in claim 54 wherein said step of isolating said oscillation comprises the step of coupling said oscillation to said sheath fluid along only one plane.

56. A system for creating a droplet from a jet of a flow cytometer comprising:

- a. a continuously converging nozzle container establishing a nozzle volume and having a nozzle exit;
- b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;
- c. a substance introduction port located within said nozzle volume; and

- d. a free fall area below said nozzle exit and within which said droplet forms.

57. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 wherein said continuously converging nozzle container comprises:

- a. a continuously converging nozzle body having an inner surface;
- b. a continuously converging nozzle tip having said nozzle exit situated thereon, wherein said continuously converging nozzle tip has an inner surface and is sealed to said nozzle body, and wherein said sheath fluid flows through said nozzle tip; and
- c. a tip joint wherein said tip joint smoothly transitions the inner surface of said nozzle body to the inner surface of said nozzle tip.

58. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 wherein said converging nozzle container continuously converges from said sheath fluid port to said nozzle exit.

59. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 wherein said nozzle container has a largest perpendicular cross sectional area and further comprising an oscillator to which said sheath fluid is responsive and which is located at said largest perpendicular cross sectional area.

60. A system for creating a droplet from a jet of a flow cytometer as described in claim 59 wherein said oscillator has an oscillator surface area and wherein said oscillator surface area is substantially as large as said largest perpendicular cross sectional area.

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61. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 and further comprising an oscillator within said converging nozzle container.
  62. A system for creating a droplet from a jet of a flow cytometer as described in claim 61 wherein said oscillator comprises a piezoelectric crystal contained within said sheath fluid.
  63. A system for creating a droplet from a jet of a flow cytometer as described in claim 62 wherein said oscillator has an oscillator surface which faces said sheath fluid, and further comprising an interface material between said oscillator surface and said sheath fluid which consists essentially of a protective coating.
  64. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 and further comprising:
    - a. an oscillator to which said sheath fluid is responsive; and
    - b. a coupling which is only planar and which couples said oscillator to said sheath fluid.
  65. A system for creating a droplet from a jet of a flow cytometer as described in claim 61 or 63 and further comprising a coupling which is only planar and which couples said oscillator to said sheath fluid.
  66. A system for creating a droplet from a jet of a flow cytometer as described in claim 64 and further comprising a directional isolator between said oscillator and said nozzle container.
  67. A system for creating a droplet from a jet of a flow cytometer as described in claim 66 wherein said oscillator emits a predominant frequency and wherein said directional isolator is effective at said predominant frequency.

68. A system for creating a droplet from a jet of a flow cytometer as described in claim 66 wherein said oscillator has an oscillator side and wherein said directional isolator comprises a spacer which maintains said oscillator side detached from said nozzle container.

69. A method of creating a droplet from a jet of a flow cytometer comprising the steps of:

- a. establishing a nozzle volume;
- b. introducing a flow of sheath fluid into said nozzle volume;
- c. converging said sheath fluid in a convergence zone;
- d. introducing a flow of a substance at a location within said sheath fluid in said convergence zone;
- e. adjusting the location at which said substance is introduced within said convergence zone;
- f. allowing said sheath fluid to exit from said nozzle volume; and
- g. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.

70. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 wherein said step of adjusting the location at which said substance is introduced within said convergence zone comprises the step of establishing the desired concentration of said substance relative to said sheath fluid.

71. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 or 70 wherein said step of adjusting the location at which said substance is introduced within said convergence zone comprises the step of establishing laminar flow of said substance within said sheath fluid.

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72. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 and further comprising the step of conducting an analysis of said droplet and wherein said step of adjusting the location at which said substance is introduced within said convergence zone comprises the step of optimizing the results of said analysis.

73. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 wherein said step of adjusting the location at which said substance is introduced within said convergence zone is automatic.

74. A method of creating a droplet from a jet of a flow cytometer as described in claim 72 wherein said step of adjusting the location at which said substance is introduced within said convergence zone comprises the steps of:

- a. sensing values representative of conditions within said flow cytometer; and
- b. automatically moving said location based upon said sensed values.

75. A method of creating a droplet from a jet of a flow cytometer as described in claim 74 wherein said conditions are at least one of the following:

- a. the pressure of said sheath fluid;
- b. the pressure of said substance;
- c. the location at which said droplet is formed;
- d. the rate at which droplet is determined to contain some of said substance; or
- e. a property of said substance.

76. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 wherein said substance is introduced through a substance tube and wherein said step of adjusting the location at which said substance is introduced within said

convergence zone comprises the step of replacing said substance tube.

77. A system for creating a droplet from a jet of a flow cytometer comprising:

- a. a nozzle container establishing a nozzle volume and having a nozzle exit;
- b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;
- c. a flow convergence zone within said nozzle volume;
- d. a substance introduction port located within said flow convergence zone;
- e. a location adjuster to which said substance introduction port is responsive;
- f. a free fall area below said nozzle exit and within which said droplet forms.

78. A system for creating a droplet from a jet of a flow cytometer as described in claim 77 wherein said substance introduction port comprises a substance tube having a fixed length and wherein said location adjuster comprises a replacement substance tube.

79. A system for creating a droplet from a jet of a flow cytometer as described in claim 77 wherein said convergence zone has a primary flow direction and wherein said location adjuster comprises a screw means which moves said substance introduction port along said primary flow direction.

80. A system for creating a droplet from a jet of a flow cytometer as described in claim 79 wherein said location adjuster comprises a telescoping substance tube which moves said substance introduction port along said primary flow direction.

81. A system for creating a droplet from a jet of a flow cytometer as described in claim 77 and wherein said location adjuster comprises:

- a. a sensor;
- b. a controller responsive to said sensor; and
- c. a movement mechanism responsive to said controller and wherein said substance introduction port is responsive to said movement mechanism.

82. A system for creating a droplet from a jet of a flow cytometer as described in claim 81 wherein said substance introduction port introduces a substance and wherein said sensor senses at least one of the following:

- a. the pressure of said sheath fluid;
- b. the pressure of a substance introduced at a location within said sheath fluid in said convergence zone;
- c. the location at which said droplet is formed;
- d. the rate at which droplet is determined to contain some of said substance; or
- e. a property of said substance.

83. A system for creating a droplet from a jet of a flow cytometer comprising:

- a. a nozzle body having an inner surface;
- b. a nozzle tip having an inner surface;
- c. a seal located off of said inner surface of said nozzle tip and to which both said nozzle body and said nozzle tip are responsive;
- d. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;
- e. a substance introduction port located within said nozzle volume; and
- f. a free fall area below said nozzle exit and within which said droplet forms.

84. A system for creating a droplet from a jet of a flow cytometer  
s described in claim 83 wherein said nozzle tip has an outer  
surface and wherein said seal contacts said outer surface of  
said nozzle tip.

85. A system for creating a droplet from a jet of a flow cytometer  
as described in claim 83 or 84 wherein said nozzle body has an  
inner surface and further comprising an edge insert on said  
inner surface of said nozzle body.